Jeremy West

Major: Chemistry
University: Truman State University
Faculty Mentor: Dr. Jack Tanner
Mentor Department: Chemistry
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Expression, purification and initial characterization of *Halobacterium* proline dehydrogenase

Jeremy West and Jack Tanner

Nature recycles proline by converting it to glutamate. This 4-electron oxidation process is catalyzed by two catabolic enzymes, proline dehydrogenase (PRODH) and Δ^1-pyrroline-5-carboxylate dehydrogenase (P5CDH). Inborn defects in PRODH and P5CDH result in the disorders hyperprolinemia I & II, respectively. These conditions are often associated with mental retardation, convulsions, and brain disorders. PRODH has also been implicated in schizophrenia susceptibility, cancer and P53-mediated apoptosis. Despite their importance in human health and disease, these enzymes have not been extensively studied. Thus, the goal of this research is to characterize the structure and function of PRODH. The work presented here focuses on a newly discovered homologue of PRODH found in archaea, which we identified by bioinformatics analysis of genome sequence data. Archaea are also genetically more closely related to eukaryotes than bacteria, so study of their proteins may provide insights into homologous eukaryotic enzymes. Archaea are some of the Earth's oldest life forms and are known for living in extreme environments. The PRODH researched here is from the *Halobacterium* (salt-loving), which can be found in places such as the Dead Sea and the Great Salt Lake. Preliminary results so far include testing the expression of *Halobacterium* PRODH, known as YusM, in two different *E. coli* expression systems, BL21(DE3)pLysS and Rosetta2. The latter strain was used to account for rare codon usage by *Halobacterium*. Parameters varied in these expression tests included time and temperature of induction as well as IPTG concentration. After expression, the cells were broken in a French pressure cell and the cell debris was pelleted with centrifugation. YusM was found to be largely associated with the cell pellet; therefore protein purification under denaturing conditions was investigated. The use of urea as a denaturing reagent has been successful for purifying YusM. Once the protein was renatured it showed improved kinetic activity. We believe the improved activity is due to disruption of improperly folded protein by the denaturant, followed by re-folding into the native, or near-native, state. Further studies will need to be done to determine the cause of misfolding in the *E. coli* cell.